REMARKS

Claims 1-10, 21 and 25-27 have been cancelled as being drawn to non-elected subject matter. Claims 11-20 have been amended and new claims 28-32 have been added. As such, claims 11-20, 22-24 and 28-32 are pending in the application.

Objection to the Title

The Title of the Invention has been objected to as not being sufficiently descriptive. The Title has been amended as suggested by the Examiner. Withdrawal of the objection is respectfully requested.

Rejection under 35 U.S.C. §101

Claims 11-18 have been rejected under 35 U.S.C. §101 as encompassing a product of nature. Claims 11-18 have been amended as suggested by the Examiner to recite, "isolated." Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §112, first paragraph

Claims 11-20 have been rejected under 35 U.S.C. §112, first paragraph for lack of written description and/or lack of enablement.

More specifically, claims 11-18 have been rejected for lacking sufficient written description for nucleic acid sequences that encode an antimicrobial protein comprising at least 50% sequence homology to SEQ ID NO:1 or for substitutions, deletions, insertions or additions thereof and for lack of enablement for nucleic acid sequences having the recited % homology. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Claim 11 is to be drawn to an isolated gene encoding an antimicrobial protein which can be obtained from a fraction of an aqueous extract of Lyophyllum shimeji precipitated by the ammonium sulfate precipitation method, wherein the protein has an antimicrobial activity at least against Rhizoctonia solani or Pyricularia oryzae, and shows the presence of components of about 70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method; or

wherein the antimicrobial protein has an amino acid sequence of SEQ ID NO:2, or has 50% or more homology with the sequence and has an antimicrobial activity against Rhizoctonia solani or Pyricularia oryzae; or

wherein the protein comprises a single polypeptide having a partial amino acid sequence of amino acid residues 76 to 618 of SEQ ID NO:2, or a polypeptide having 50% or more homology with the partial amino acid sequence and having an antimicrobial activity

against Rhizoctonia solani or Pyricularia oryzae, or a combination of these polypeptides.

The gene encoding the antimicrobial protein of claim 11, and all dependent claims thereon, is both structurally and functionally defined in the claims. The antimicrobial protein encoded by the gene of the invention is defined structurally as being obtained from a fraction of an aqueous extract of Lyophyllum shimeji precipitated by the ammonium sulfate precipitation method, and having components of about 70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method. Alternatively, the protein has been structurally defined as having having a partial amino acid sequence of amino acid residues 76 to 618 of SEQ ID NO:2 or 50% or more homology with the partial or entire amino acid sequence of SEQ ID NO:2. The antimicrobial protein has been further defined by having antimicrobial activity against Rhizoctonia solani or Pyricularia oryzae. As such, the invention is adequately described, such that one skilled in the art would know whether a particular gene encoding an antimicrobial protein fell within the claims. In addition, based on techniques disclosed in the specification and well-known at the time of the invention, one skilled in the art would be readily able to isolate and identify genes encoding proteins that fall within the scope of the claims.

Applicants further note that the present invention is drawn to a completely novel gene encoding an antimicrobial protein. There are no known genes that having a high degree of homology to the gene of the present invention. As such, the present inventors have achieved a breakthrough invention that should be given the broadest protection possible.

Claims 19 and 20 have been further rejected for the recitation in claim 19 of optionally modifying the single-stranded DNAs while avoiding damage to the binding specificity to the base sequence of said gene encoding the antimicrobial protein. The Examiner indicates that there is no discussion as to what is meant by the modifications. Applicants traverse this rejection and withdrawal thereof is respectfully requested. One skilled in the art would readily know that "modification" means the replacement of one or a few bases from SEQ ID NO:1, without the loss of activity.

Claims 19 and 20 have been further rejected for being drawn to an oligonucleotide used for obtaining a gene comprising "at least 50% homology to SEQ ID NO:1." It appears from the rejection, that the Examiner has mistakenly read claim 19 as depending from claim 11. The Examiner appears to have interpreted claim 19 as using, as a starting sequence for the oligonucleotides, a gene having 50% homology to SEQ ID NO:1. However, claims 19 is an independent

claim that recites, in part, the use of SEQ ID NO:1 as a base sequence, wherein two domains from the gene of SEQ ID NO:1 are selected such that 1) each domain consists of 15 to 30 bases; and 2) each domain has 40 to 60% of G+C. As such, the invention of claims 19 and 20 is fully described and enabled as written.

Rejections under 35 U.S.C. §112, second paragraph

Claims 11-18 and 22-24 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. More specifically, claim 11 has been rejected for depending from a non-elected claim. Claim 11 has been amended to be in independent form.

Claim 19 has been rejected for containing a typographical error in the word "form" in line 5 and as being unclear as to what minimal sequence is needed. Claim 19 has been amended to correct "form" to "from." With regard to the minimal sequence, Applicants note that claim 19 recites that 1) each domain consists of 15 to 30 bases; and 2) each domain has 40 to 60% of G+C. Thus, the "minimal" sequence is defined in claim 19.

Claim 12 has been rejected for being indefinite regarding "stringent conditions." The Examiner asserts that the example on page 16, lines 17-21 is not considered definitive. Claim 12 has been amended to incorporate the stringency conditions from the body

of the specification into the claims. As such, the hybridization conditions have been fully defined.

Claim 12 has been further rejected for recitation of a sequence that hybridizes to SEQ ID NO:1, based on the position that a hybridizing sequence would not encode the same protein. Claim 12 has been further rejected for having improper Markush group format. Claim 12 has been amended to clarify that the protein is encoded by a sequence complementary to the hybridizing sequence and to recite proper format.

As the above-comments and amendments address and overcome the objections and rejections, withdrawal of the objections and rejection and allowance of the claims are respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, PhD (Reg. No. 40,069) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

A marked-up copy of the amended portions of the specification and claims is attached hereto.

Applicants request a three (3) month extension of time for filing the present response. The required fee is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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MARKED-UP VERSION SHOWING CHANGES

IN THE TITLE

The Title of the Invention has been amended as follows.

~~--A NOVEL PROTEIN, A GENE CODING THEREFOR AND A METHOD OF USING THE SAME~~ **ANTIMICROBIAL PROTEIN FROM *LYOPHYLLUM SHIMEJI***--

IN THE CLAIMS

Claims 1-10, 21and 25-27 have been cancelled.

Claims 11-20 have been amended as follows.

11. (Amended) ~~A~~ **An isolated** gene encoding an antimicrobial protein ~~according to Claim 1, 3 or 9~~

which can be obtained from a fraction of an aqueous extract of Lyophyllum shimeji precipitated by the ammonium sulfate precipitation method, wherein said protein has an antimicrobial activity at least against Rhizoctonia solani or Pyricularia oryzae, and shows the presence of components of about 70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method; or

wherein said antimicrobial protein has an amino acid sequence of SEQ ID NO:2, or has 50% or more homology with said sequence and

has an antimicrobial activity against Rhizoctonia solani or Pyricularia oryzae; or

wherein said protein comprises a single polypeptide having a partial amino acid sequence of amino acid residues 76 to 618 of SEQ ID NO:2, or a polypeptide having 50% or more homology with said partial amino acid sequence and having an antimicrobial activity against Rhizoctonia solani or Pyricularia oryzae, or a combination of these polypeptides.

12. (Amended) The **isolated** gene according to Claim 11**, encoding an antimicrobial protein and** having a base sequence of SEQ ID NO:1 ~~in the Sequence Listing, a base sequence derived from said sequence by substitution, deletion, insertion and/or addition of one or more bases~~, or a base sequence **which is complementary to a base sequence which hybridizes to SEQ ID NO:1** ~~capable of hybridizing to said base sequence(s)~~ under stringent conditions **of 6 x SSC, 45°C to 68°C (without formamide) or 25°C to 50°C (with 50% formamide)**.

13. (Amended) The **isolated** gene according to Claim 11 **encoding a protein having antimicrobial activity and** having a 50% or more homology with the base sequence of SEQ ID NO:1 ~~in the Sequence Listing~~.

14. (Amended) The **isolated** gene according to Claim 11 **encoding a protein having antimicrobial activity and** having a 60% or more homology with the base sequence of SEQ ID NO:1 ~~in the Sequence Listing~~.

15. (Amended) The **isolated** gene according to Claim 11 **encoding a protein having antimicrobial activity and** having a 70% or more homology with the base sequence of SEQ ID NO:1 ~~in the Sequence Listing~~.

16. (Amended) The **isolated** gene according to Claim 11 **encoding a protein having antimicrobial activity and** having an 80% or more homology with the base sequence of SEQ ID NO:1 ~~in the Sequence Listing~~.

17. (Amended) The **isolated** gene according to Claim 11 **encoding a protein having antimicrobial activity and** having a 90% or more homology with the base sequence of SEQ ID NO:1 ~~in the Sequence Listing~~.

18. (Amended) The **isolated** gene according to Claim 11 **encoding a protein having antimicrobial activity and** having a 95%

or more homology with the base sequence of SEQ ID NO:1 ~~in the Sequence Listing~~.

19. (Amended) An oligonucleotide for obtaining a gene encoding an antimicrobial protein originated from Lyophyllum shimeji produced by a process comprising:

selecting two domains ~~satisfying the following requirements form~~ **from** the base sequence of the gene ~~encoding the antimicrobial protein~~ of SEQ ID NO:1 **wherein said domains satisfy the following requirements** ~~in the Sequence Listing~~:

1) each domain ~~consisting~~ **consists** of 15 to 30 bases; and

2) each domain ~~having~~ **has** 40 to 60% of G+C;

preparing single-stranded DNAs having base sequences which are identical to the base sequences of said domains or complementary thereto, or preparing a single-stranded DNA mixture having degeneracy in the genetic code which ensures that the amino acid residues coded by said single-stranded DNAs are not changed; and optionally modifying the single-stranded DNAs while avoiding damage to the binding specificity to the base sequence of said gene encoding the antimicrobial protein.

20. (Amended) The oligonucleotide according to claim 19 having a nucleotide sequence of any one of SEQ ID **NOS:7 to 12** ~~Nos:7 to 12 in the Sequence Listing~~.

New claims 28-32 have been added.